

(a) isolating, chemically synthesizing or amplifying with polymerase chain reaction (PCR) a cDNA, mRNA or genomic DNA encoding a light or heavy chain of the antigen-specific antibodies and assembling the antibodies cDNA encoding said light and heavy chains of said antibodies into two separate expression cassettes, one encoding DNA for the light chain and the second encoding DNA for the heavy chain, each cassette further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5' terminus and by the yeast transcription termination DNA sequence of the 3'-terminus,

6, 6A wherein the antibody cDNA is assembled into the expression cassettes by subcloning the antibody light and heavy chain cDNA in tandem as *EcoRI*-*BglIII*/*BsmBI* fragments flanked by a DNA encoding the *P. pastoris* signal sequence, preceded by a *P. pastoris* promoter at the 5'-terminus and by a *P. pastoris* yeast transcription termination DNA sequence at the 3'-terminus, and

wherein the signal sequence is a yeast  $\alpha$ -factor and wherein the promoter is an alcohol oxidase AOX1-P;

(b) preparing a recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector pPICZ $\alpha$  by restriction digestion with *EcoRI* and *BamHI*;

(c) constructing a recombinant *P. pastoris* yeast expression plasmid containing the expression cassettes of step (a);

(d) cloning the expression cassettes of step (c) into the *P. pastoris* expression vector pPICZ $\alpha$  to generate recombinant plasmid pPICZ $\alpha$ LH comprising expression cassettes for the light and heavy

chains;

(e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes of step (d) under the control of the AOX1 promoter fused to the DNA encoding the *Saccharomyces cerevisiae*  $\alpha$ -mating factor signal;

(f) amplifying and isolating the recombinant plasmid;

(g) transforming *P. pastoris* spheroblasts with *Bgl*II linearized, *Not*I linearized, *Sac*I linearized, *Sal*I linearized or *Stu*I-linearized recombinant plasmid replacing the yeast chromosomal AOX1 DNA sequence with AOX1-antibody DNA sequence containing expression cassettes of the recombinant plasmid of step (d);

(h) selectively growing the recombinants;

(i) screening yeast transformation colonies for a recombinant antibody expression;

(j) analyzing putative positive yeast clones for chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;

(k) confirming the integrity of the DNA insert;

(l) inducing the recombinant antibody expression;

(m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;

(n) detecting the presence of the recombinant antibody by Western blot;

(o) testing the recombinant antibody for specific antigen-antibody binding, and

G1  
G2  
(p) harvesting the antigen-specific antibody produced in steps (a) - (o);

wherein said antibody is produced in quantity of 10-36 mg/l in about 12 to about 108 hours.

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G2  
25. (Amended) The method of claim 22 wherein the antigen is dioxin.

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G3  
27. (Amended) The method of claim 22 wherein the replacement of the yeast chromosomal AOX1 with AOX1-antibody cDNA containing cassettes is by homologous recombination replacement.

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#### REMARKS

This Amendment is filed in Response to the Final Office Action dated June 20, 2001 wherein Examiner rejects all pending claims 22-35 in a Continued Prosecution Application (CPA) for the reasons of record.

#### Reiteration of the Invention

The following is a brief reiteration of the invention and its main points.

The method of the invention produces an entire intact antibody, that is it produces an exact replica of the whole antigen-specific antibody, raised against the specific antigen by constructing two expression cassettes corresponding to, and each comprising, the light and/or heavy chains DNAs of the target antibody. The cassettes are flanked by a signal sequence preceded